International Journal of Veterinary Science and Medicine (2014) 2, 142–150

HOSTED BY

ELSEVIER

Cairo University

International Journal of Veterinary Science and Medicine

www.vet.cu.edu.eg
www.sciencedirect.com


Full Length Article

# Iodoacetate and allogenous cartilage particles as models for arthritis induction in equine


**Ahmed Elmesiry** [a,*], **Magdi Seleim** [b], **David Cullis-Hill** [c]

[a] *Animal Reproduction Research Institute, Agriculture Research Center, 12556 Haram, Giza, Egypt*
[b] *Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Kafrelsheikh University, 33156 Kafrelsheikh, Egypt*
[c] *Biopharm Pharmaceuticals, 111 Bronte Rd, Bondi Junction, Sydney, 2022 NSW, Australia*

Received 22 August 2014; revised 29 October 2014; accepted 7 November 2014
Available online 4 December 2014

**KEYWORDS**

Equine;
Lameness;
Joint;
Cartilage;
Osteoarthritis

**Abstract** Experimental models of osteoarthritis (OA) have been widely developed in different animal species, because of the high incidence of osteoarthritis diseases in humans and animals. To date, no ideal OA animal model has been reported. The present study compare different osteoarthritis models to determine which one is suitable for inducing experimental equine OA. Fifteen donkeys were divided into three equal groups ($n = 5$). The radio carpal joints of the right forelimb of 15 donkeys were injected with 25 mg monoiodoacetate (MIA) (group A), 50 mg allogenous cartilage particles (ACP) (group B), or vehicle solution (group C) over a period of 70 days. Osteoarthritis induction was evaluated weekly through lameness score, carpal circumference, joint flexion angel, synovial fluid analysis (total protein and WBC count), and radiology. Animal were euthanized and joints histopathology were performed at 70 days. Lameness score and joint circumference was increased in both group A and B however joint flexion angel was decreased compared to group C ($p < 0.05$). Osteophytes were observed in MIA injected joints only accompanied with subchondral bone sclerosis. Cartilage damage was observed grossly and histologically in Group A together with synovial membrane fibrosis. Group B had on cartilage damage grossly however histological examination revealed some cartilage surface discontinuity with synovial membrane edema. Injection of monoiodoacetate in the donkey is a successful model to create the acute clinical signs of joint disease as well as cartilage damage. However, allogenous cartilage particles injection need more investigation to be applied.



## 1. Introduction

In the equine industry, lameness due to joint disease is the most common cause of decreasing the performance in sport horses. Several epidemiologic studies have found that lameness due to joint disease is the most significant factor responsible for inability to race and loss of performance [1,2]. Therefore,

* Corresponding author. Tel.: +20 1062099431.
E-mail address: elmesiry@outlook.com (A. Elmesiry).
Peer review under responsibility of Faculty of Veterinary Medicine, Cairo University.

http://dx.doi.org/10.1016/j.ijvsm.2014.11.001

it is important to understand the pathogenesis and medications available to the equine practitioner.

Equine osteoarthritis (OA) may be considered as a group of disorders characterized by a common end stage: progressive degeneration of the articular cartilage together with additional changes in the bone and soft tissues of the joint. This degeneration of the articular cartilage is characterized by local splitting and fragmentation (fibrillation) of articular cartilage. Synovitis and joint effusion are often associated with the disease, and, clinically, the disease is characterized by pain and dysfunction of the affected joint [3].

Animal models are standard research tools for studying the pathogenesis, diagnosis and potential therapeutic intervention of many different diseases. They provide us with information to develop new drugs and moving it toward clinical use. The different types of arthritis models have been previously reviewed [4–7].

Common features of most experimentally-induced osteoarthritis models include the ability to define the type of joint disease, the severity of injury in addition to the time of onset and progression and to relate these events to markers of disease activity [4].

Arthritis-like changes have been induced in the horse by Filipin [8], Amphotericin [9,10], turpentine oil [11], polyvinyl alcohol foam [12], carrageenan [13], complete Freund's adjuvant [14]. Lipopolysaccharide [15], botulinum toxin [16], forced exercise [17], osteochondral fragment-exercise mode [18].

The monoiodoacetate (MIA) arthritis model has been used in rats [19], chickens [20], guinea pigs [21], rabbits [22] and horses [23–25] for assessment of the pathophysiologic process as well as evaluation of the efficacy of therapeutic substances in a controlled environment.

Using cartilage particles to induce osteoarthritis was previously described in dog [26] and rabbit [27]. A combination of intra-articular injection of cartilage particles, arthroscopic partial thickness cartilage defect and exercise were used to create a model of degenerative joint disease in the horse [28]. The fate and effects of surgically implanted osteochondral fragments on the middle carpal joint of horses subjected to exercise were investigated [29].

The donkey is properly the closet animal to the horse, making this species an alternative animal model for studying equine diseases. Few papers reported using of donkey as a model of equine OA [10,11].

In the present study, injections of allogenous cartilage particles (ACP) or monoiodoacetate (MIA) were used to create a model of degenerative joint disease in the donkey. The clinical examination, radiographic, macroscopic appearance, and light microscopy were used to assess the effect of these treatments on healthy cartilage compared to the vehicle control.

## 2. Material and methods

### 2.1. Donkeys

The experiment was approved by the Committee on Animal Experimentation at the Kafrelsheikh University, Egypt.

The present study was performed using 15 healthy Egyptian local breed male donkeys weighting from 150 to 200 kg. Animals were housed in indoor stalls and fed on a balanced ration of mixed grain with hay and unlimited water. All donkeys were dewormed with ivermectin (200 mcg/kg; Eqvalan 1.87% Merial Limited. USA).

Prior to inclusion in the study, lameness examination, body condition, radiographs of carpal joints, range of motion of carpal joints (angle of flexion) and evidence of joint effusion were assessed to ensure that all previous variables were within normal limits (baseline measurement).

Donkeys were allowed to acclimatize for 2 weeks prior to the study. During the acclimatization period, the donkeys trained daily to familiarize them to the experimental conditions (investigators, environment, handling, vein puncture and various outcome measures).

### 2.2. Allogenous cartilage particle solution preparation (ACP)

One local breed donkey weight 150 kg was euthanized, and the articular cartilage was removed from the shoulder, carpal, fetlock, pastern, hock and stifle joints in a biosafety cabinet under aseptic conditions. The pooled cartilage was powdered under liquid nitrogen in a mortar, producing particles as small as 20 mm in diameter (able to pass easy through a 14-gauge needle). These particles were resuspended at a concentration of 50 mg/ml in a physiological saline solution contained amikacin sulfate (50 mg/ml; Amikin 500 mg vial, Bristol Meyer Squiip, Egypt).

### 2.3. Monoiodoacetate solution preparation (MIA)

MIA (Sodium monoiodoacetate 25 g, ICN, Biomedicals GmBH Thuriger star be 15.Germany) were dissolved at a concentration of 25 mg/ml in a physiological saline solution contained amikacin sulfate (25 mg/ml).

### 2.4. Study design

The 15 remaining donkeys were divided in to three groups of five. Animals sedated with Xylazine Hcl (1 mg/kg; Rumpon 10%, Bayer animal health. Canada). The skin was aseptically prepared for arthrocentesis of each right radiocarpal joint to obtain synovial sample for baseline analysis. Group A received 25 mg (1 ml solution) of MIA, Group B received 50 mg (1 mL) of ACP and Group C was received the suspended solution (1 mL) without adding cartilage or MIA (Vehicle – Control group) intra-articularly into the right radiocarpal joint using a 14 G needle. These injections were repeated at 7, 14, 21, 28, 35, 42 and 56 days for group B and C however Group A was received a single MIA injection (Fig. 1).

### 2.5. Outcome measures

#### 2.5.1. Clinical examination

Clinical examinations of right forelimbs were performed weekly from day 0 (baseline) throughout the study period.

#### 2.5.2. Lameness score

Donkeys evaluated for lameness score on a scale 0–5 according to American Association of Equine Practitioners (AAEP) grading system (0: Lameness not perceptible with flexion test, 1: lameness is difficult to observe and is not consistently

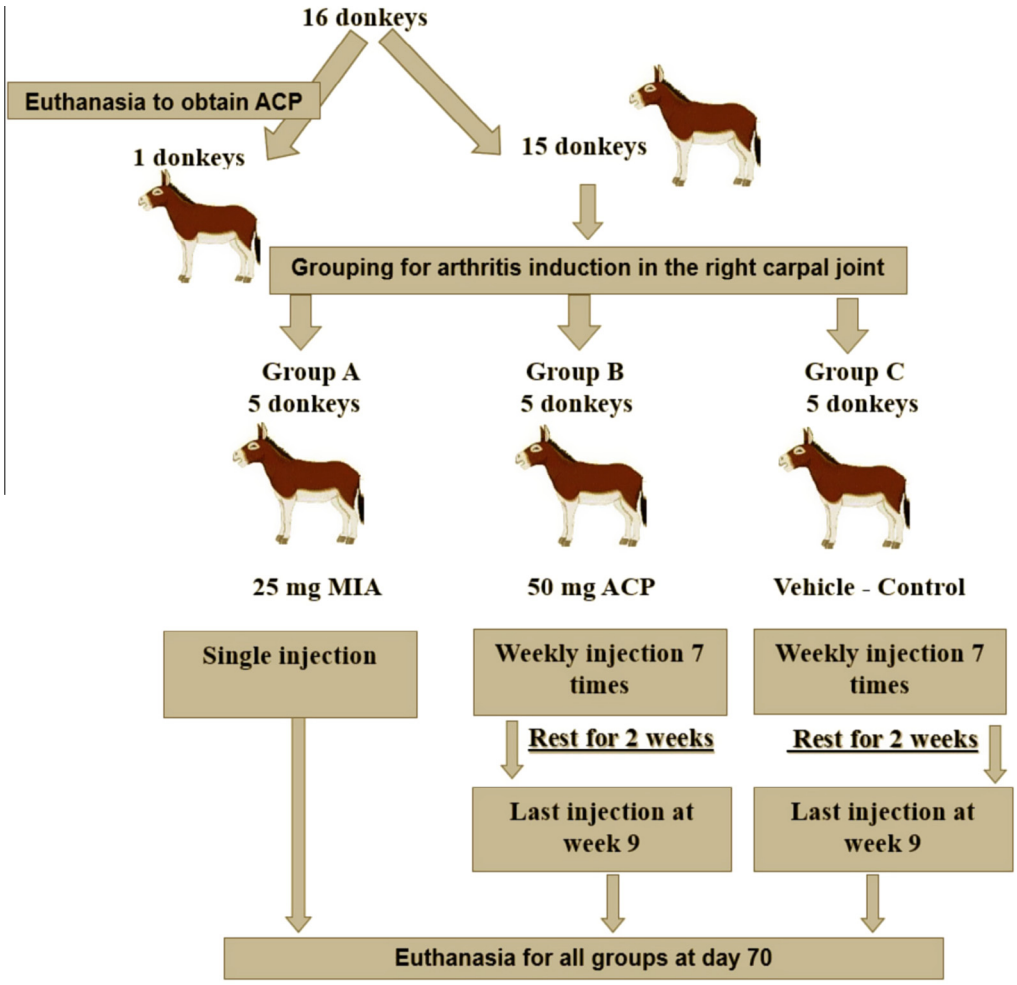


**Figure 1** Flowchart of the study protocol.

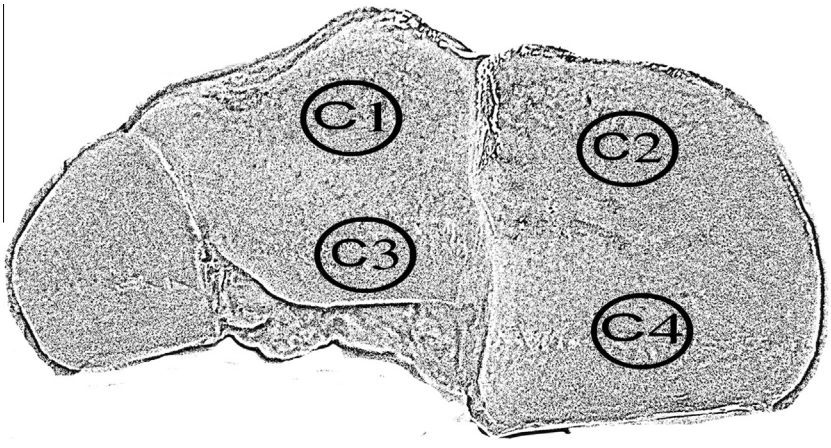


**Figure 2** Illustration of cartilage collection sites from distal articular surface of the radius.

apparent with flexion test, 2: lameness apparent with flexion test, 3: lameness is consistently observable at a trot, 4: lameness is obvious at a walk, 5: lameness produces minimal weight bearing in motion) [30].

*2.5.3. Circumference of the carpal joint*

Measurements obtained at the proximal aspect of the carpus by using of a measurement tape (in cm), and with the aid of the anatomical reference points (accessory, radial, ulnar and

intermediate carpal bones). Circumference was obtained weekly; hair over the selected area was clipped on a regularly scheduled basis [14].

#### 2.5.4. Maximum carpal flexion angel

Maximum carpal flexion was measured weekly by slowly flexing the carpus until the donkey resisted. The angle was then measured in degrees with Goniometer [14].

#### 2.5.5. Synovial fluid analysis

Synovial fluid sample (1 mL) aseptically aspirated from each joint before each injection. The conventional analysis of synovial fluid included assessment of total protein concentration, white blood cell (WBC) count, and differential count. Total protein concentrations and WBC were determined via Double Beam UV Visible Spectrophotometer and use of an automated cell counter, respectively [18].

#### 2.5.6. Radiographic evaluation

Radiographic evaluation of both carpi was performed prior to inclusion in the study (day 0), following the induction of osteoarthritis (day 7), and weekly to the end of the study (day 70). The radiographs were taken in dorsopalmar view and a professor of radiology assessed images blindly without known of the treatment group. The radiographic images were evaluated categorically for bony proliferation at the joint capsule attachment, subchondral bone sclerosis, and osteophyte formation on a scale of 0–4 (0 represented normal, and 4 represented severe change) [18].

#### 2.5.7. Gross pathology of joint tissue

All donkeys were euthanized at day 70 by administration of pentobarbitone sodium (100 mg/kg IV; Nembutal Sodium 5%. Lundbeck Inc. Deerfield, IL 60015. USA) and tissue samples collected from joint capsule and articular cartilage (Fig. 2). Carpal joints specifically examined for degree and location of articular cartilage fibrillation or erosion. A subjective grade (scale of 0–4) assigned for partial- and full-thickness cartilage erosion as well as synovial membrane hemorrhage. A total erosion score assigned, also with a scale of 0–4. For each of the 2 variables, grade 0 represented no pathological change and 4 represented a severe change [31].

#### 2.5.8. Light microscopy

Specimens from the synovial membrane and joint capsule harvested and placed in neutral-buffered 10% formalin (NBF), stained with H&E and examined microscopically. Samples evaluated for cellular infiltration, synovial intimal hyperplasia, subintimal edema, subintimal fibrosis and subintimal vascularity. Each variable was graded and reported as a numeric value 0–4 (0 = normal, 1 = slight change, 2 = mild change, 3 = moderate change, and 4 = severe change [31].

Full thickness articular cartilage samples of 5-mm$^2$ diameter were Obtained from each joint (Fig. 1 – shows squares). Sampling sites chosen to represent an area of thick cartilage (C1 & C2) and thin cartilage (C3 & C4) regions. Samples placed in (NBF) for 7 days and then placed in 10% EDTA for 21 days for decalcification then processed routinely to paraffin wax for histological. Samples were sliced into 5-μm sections and stained with H&E, cartilage were graded on a scale of 0–6 (Grade 0: smooth, grade 1: surface irregular, grade 2: surface discontinuous, grade 3: vertical fissure, grade 4: erosion, grade 5: denudation, grade 6: deformation) [32] (Fig. 2).

### 2.6. Statistical analysis

Variables including lameness, carpal flexion angel, carpal circumference, TP and TWBC analyzed using a repeated measures analysis of variance (ANOVA) model with SPSS (Version 17: WinWrap Company release 2008). Any test with a $p$ value $<0.05$ was declared statistically significant. When individual comparisons were made, Bonferroni post hoc test was used and $p < 0.05$ was considered significant. The Kruskal–Wallis non-parametric ANOVA was used to evaluate statistical differences in gross pathological and histo-pathologic scores. Values are reported as mean ± Standard deviation.

## 3. Results

### 3.1. Clinical examination

#### 3.1.1. Lameness score

Increasing in the lameness score begin at day 7 for group A (2 ± 0), and at day 14 for group B (0.6 ± 0.49) and peak at day 14 for group A (2.4 ± 0.49) and at day 21 for group B (1.6 ± 0.49). At day 56, the lameness score was at lowest value for group A (1 ± 0) and B (0.4 ± 0.49). Group C had no lameness all over the study period (Fig. 3-A).

There was a significant change between group A groups C ($p < 0.001$) at each study day. However, group B was significantly different from group C at day 21, 28, 35 and 42. Group A was significantly difference from group B at days 7, 14 and 42 ($p < 0.05$).

#### 3.1.2. Carpal circumference

Circumference in group A was strongly increased at day 7 (24.88 ± 0.55) then begin decline till the end of the study. However, Group B was smoothly increased reached the peak at day 35 (23.92 ± 0.48) then begin to decrease until the study end. Group C was weakly increased but not significantly changed (Fig. 3-B). Group A was significantly difference from group C at each study day ($p < 0.05$) and was significantly difference from group B at day 7, 14, 21, 28, 56 and 70. However, group B and C were not significantly different from each other ($p > 0.05$).

#### 3.1.3. Maximum carpal flexion angel

The flexion angel suddenly decreased at day 7 in-group A (106 ± 3) then begin to decrease smoothly until the end of the study. Group B decreased smoothly until reach the peak at day 35 (141 ± 2) then begin to decrease to the end of the study. Group C had a constant flexion angle throughout the study period (Fig. 3-C). Group A was significantly difference from group B and C on each study day ($p < 0.05$). However, group B was significantly different from group C from day 21 until day 42.

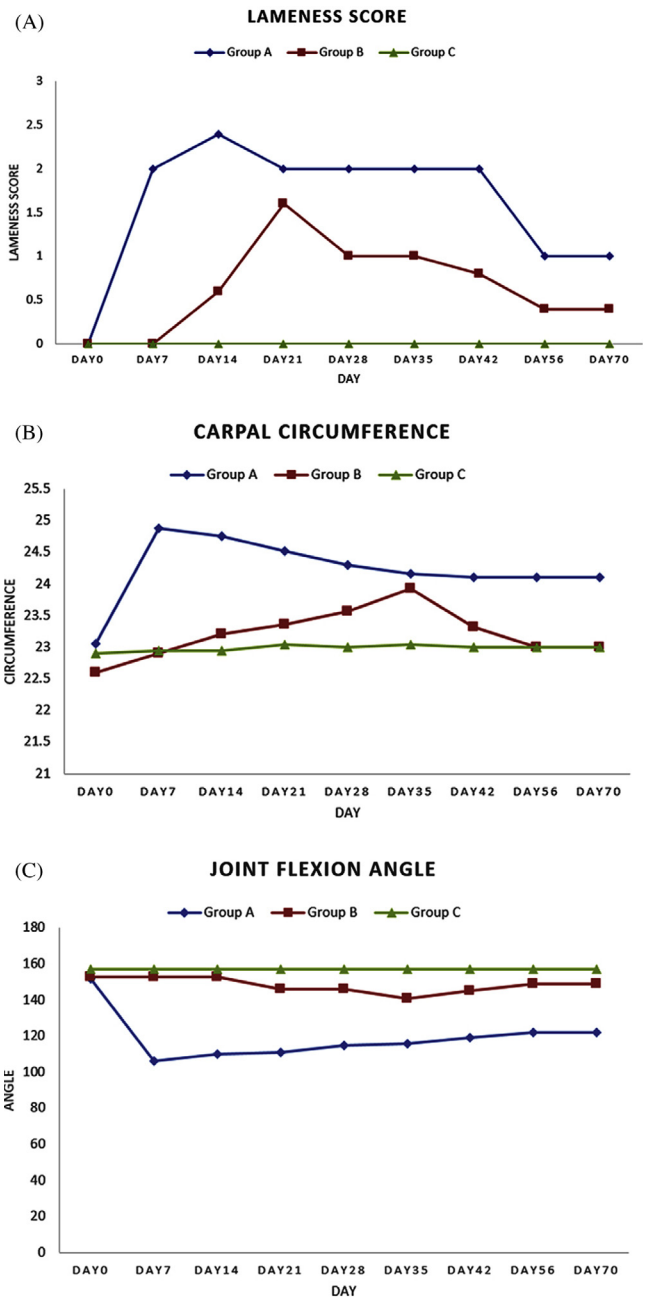


**Figure 3** (A) Main lameness score over the study period. (B) Main Carpal Circumference over the study period. (C) Main carpal flexion angle over the study period.

### *3.1.4. Synovial fluid analysis*

Synovial WBC count was increased at day 7 in all groups. Group A reached its peak at day 7 (874 ± 72), group B at day 35 (620 ± 74) and group C at day 42 (360 ± 81).

Group A was significantly difference from group B and C on each study day ($p < 0.05$). However, group B was significantly different from group C on study day 14, 21, 28, and 35.

Total protein also increased in Group A, Group B and Group C after the first injection throughout the study. Group A was significantly difference from group B and C ($p < 0.05$) but Group B and C were not significant difference from each other.

### *3.1.5. Radiographic analysis*

At day 70 group B and C treated joints had no radiographic changes while Group A treated joints of all donkeys had grade 4 radiographic score with narrowing of joint space and secondary features as osteophytes formation, and subchondral bone sclerosis (Fig. 4). There was a significant ($p < 0.0001$) difference between group A and the other groups.

### *3.1.6. Gross pathology*

Group A had partial and full thickness erosion (Mean 2.67 ± 0.47) with a significant difference from group B & C ($p = 0.023$). However, group B & C had no gross fibrillation or fissuring (Mean 0 ± 0) and were thus not different from each other (Fig. 5). The synovial membrane hemorrhage had significantly difference between the three groups ($p = 0.047$). Group A was significantly deference from group B ($p = 0.034$) and group C ($p = 0.001$). Group B & C also were not significantly deference from each other ($p = 0.114$).

### *3.1.7. Light microscopy of synovial membrane*

Induction of osteoarthritis did not result in significant change in synovial membrane intimal hyperplasia or subintimal edema ($p = 0.139$). Group A&B had slight changes (1 ± 0).

Synovial membrane cellular infiltration was increased in Group A (3.33 ± 0.47), and group B (1 ± 0). Group A was significantly different from group B ($p = 0.034$). Group A characterized by large cartilage particles embedded inside the subintimal layer surrounded by a sever zone of cellular infiltration (Fig. 6).

Synovial membrane vascularity was increased in Group A (2.67 ± 0.47) and group B (0.66 ± 0.47). Group A was significantly different from group B ($p = 0.034$).

Synovial membrane fibrosis was increased in Group A (2.67 ± 0.47). Group A was again significantly different from Group B & C ($p = 0.034$). However Group B & C were not different ($p = 0.099$).

### *3.1.8. Light microscopy of articular cartilage*

Histologic evaluation of sample C1 via H&E revealed a significant increase in OA score for Group A from Group B ($p = 0.043$) and Group C ($p = 0.034$). There was a significant difference between Group B and Group C ($p = 0.034$). C1 & C2 histologic score revealed more damage than C3 & C4 although this difference was not significant ($p = 0.518$). Lesion on In Group A (C1: 3.67 ± 0.47 & C3: 2.67 ± 0.47), however, vertical fissures (matrix vertical fissures into mid zone) and erosion (superficial layer and mid zone lost their matrix) were the most common (Fig. 7A). Group B (C1: 1.67 ± 0.47 & C3: 0.67 ± 0.47), suffered from matrix discontinuity at superficial zone, disorientation of chondron columns and cell proliferation (clusters) (Fig. 7B).Group C revealed normal architecture and, appropriate orientation of the Cells (Fig. 7C).

## 4. Discussion

Experimental models of osteoarthritis (OA) have been widely developed in different animal species Because of the high incidence of osteoarthritis diseases in humans and animals, however none of the existing models perfectly resemble the natural disease [6].

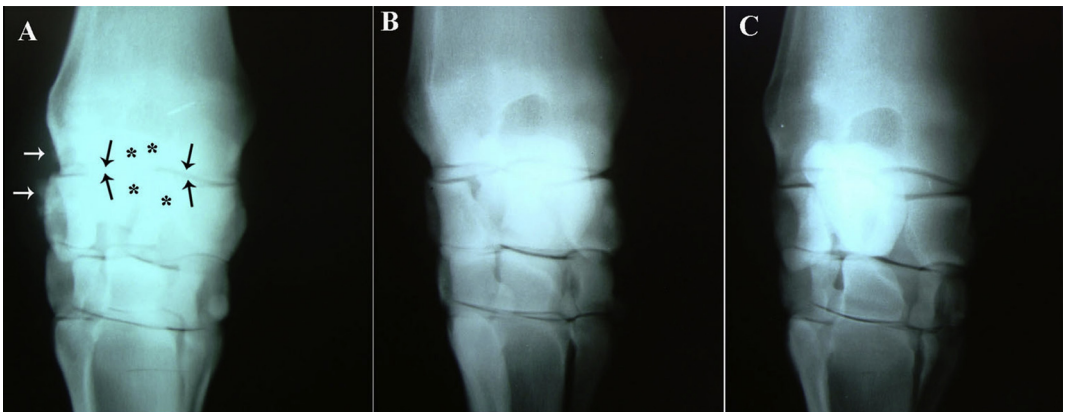


**Figure 4** Representative images of the dorsopalmar radiographs of the carpal joint of Group A, B, & C. white arrows refer to osteophytes, black arrows refer to thinning of articular cartilage however, and the black asterisks refer to subchondral bone sclerosis.

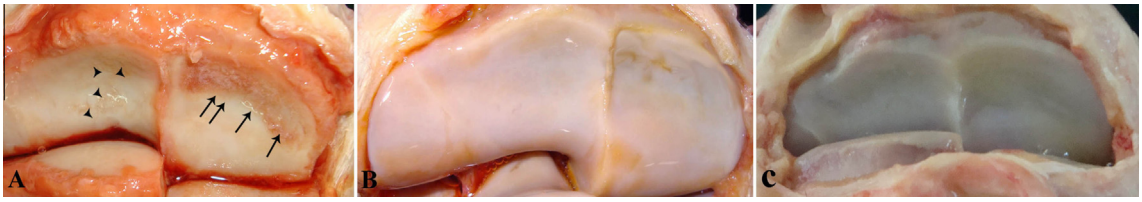


**Figure 5** Representative images of the gross morphology of the distal articular surface of the radius of Group A, B, & C. Black arrows refer to full thickness cartilage erosion however, arrows head refer to partial thickness cartilage erosion.

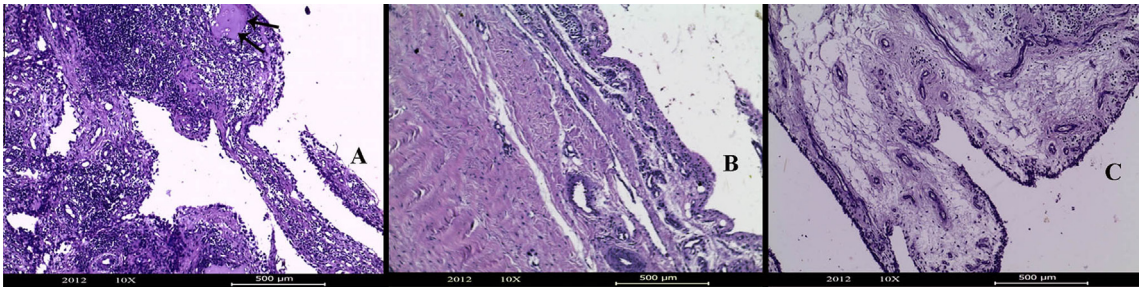


**Figure 6** Representative images of the light microscopy image of the synovial membrane of the three groups A, B and C. Black arrows refer to cartilage fragments. H & E stain 10×.

In the present study, intraarticular injection of allogenic cartilage particles or monoiodoacetate effectively resulted in clinical, histologic, and biochemical changes indicative of osteoarthritis. Lesions are vary from mild osteoarthritis with superficial cartilage fibrillation to moderate osteoarthritis with cartilage erosion. During this study, no adverse events were recorded with any of the treatment doses and a mild degree of lameness was induced.

MIA act by inhibiting the glyceraldehyde-3-phosphate dehydrogenase activity in the chondrocytes (an inhibitor of glycolysis) promotes loss of articular cartilage similar to that noted in human OA [19]. In the absence of an effective treatment, the model will induce pathological detectable features of OA [25].

The mechanisms of action of ACP to create cartilage erosion is controversial, but probably occurs by two mechanisms. First, cartilage particles may initially act as abrasives to liberate more 'wear particles' which are engulfed by the synovial membrane [33,34] causing metaplastic changes in the membrane, abnormal synovial fluid constituents and synovitis. Secondly, cartilage particles may also stimulate synovial cells via immune system to produce cellular mediators and/or proteinases which result in depletion of cartilage matrix, either directly or by acting on the chondrocytes [35].

To study osteoarthritis in horses we should use an animal model phylogenetically, biomechanically and biochemically close as is possible in order to most accurately reflect pathologic process in the target species [7]. Thus for horse a donkey model is likely to be superior to a dog, rabbit or mouse model. Donkey has many benefits that give him the opportunity to become a good alternative for the horse including low animal price, feed stuff and animal housing, in addition to large joint sample for histopathology.

In the horse, complete surgical transection of the cranial cruciate ligament in horses has not resulted in progressive

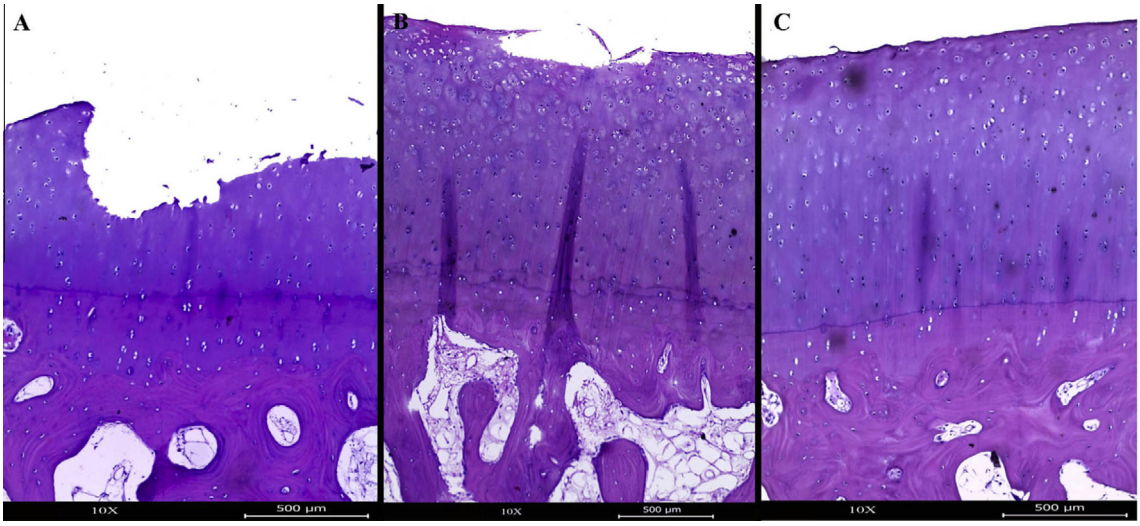


**Figure 7** Reprehensive images of the light microscopy image of the articular cartilage (C1) of the three groups A, B and C. H & E stain 10×.

OA like other species [5]. Carrageenan [13] and Polyvinyl alcohol foam [12] induces transient lameness, but only for a short period. Application of Escherichia coli lipopolysaccharide (LPS) [15], Amphotericin [9] or the polyene antibiotic filipin [8], Freund's complete adjuvant [14] are well-described models, but use of either can result in severe, non-weight-bearing lameness, induce irreversible osteophytosis and articular lesions. In addition, all these agents can result in substantial increase in the circumference of the joint, compared with that of the control joint, owing to severe inflammatory response in the periarticular tissues.

In the osteochondral fragment models, the animals are subjected to very invasive surgery with postoperative antibiotics and non-steroidal anti-inflammatories that may affect the function of the body system. In addition, induction of the chondral defects lack the natural sequence of the disease process.

Single MIA dose used upon previous work in horse where 0.16 mg/kg was used [25]. ACP dose was made upon previous work with rabbit where 1 mg/kg was used [27]. Repeated injection of ACP is needed to accelerate a natural degenerative process that normally takes years to develop.

Lameness is often a feature of natural or experimentally induced OA. In some cases, lameness may be severe that limits usefulness of a model [8]. Despite the fact that articular cartilage degeneration was induced in our study, lameness was a major feature of MIA group in contrast to the ACP groups.

In the ACP group, carpal circumference and joint flexion angel changes were changed smoothly in contrast to MIA group in which changes are strongly specially after the first injection. Rapid response to the MIA injection resemble the acute traumatic joint disease with local pain on palpation. This giving the MIA the priority to be a good model for acute synovitis.

Increase synovial fluid WBC and TP in MIA at day 7 is indicative for the acute synovitis and confirm the evidence of clinical signs of lameness and pain. However in ACP group, response is delayed to day 14 without increase in TP which may be due to low cartilage dose to initiate inflammatory response.

Periarticular osteophyte and narrowed joint spaces were a significant feature in MIA injected joints. This is in opposite to other studies using MIA joint disease models [23,24] however, absence of radiographic changes with ACP was in contrast to other studies by Evan [27] in which presence of osteophytes in the knee joints of one group of rabbits after 4 months of cessation of injections.

Gross cartilage degeneration is confirming the narrowing on the joint space on the radiographic film on MIA group. Additionally the synovial membrane hemorrhage is indicative for the inflammatory process which happen on the synovial membrane.

In our study superficial cartilage fibrillation were observed on the ACP group, this confirm the previous study in the horse where the lesions were limited to wear lines and areas of cartilage thinning together with capsular fibrosis and synovial membrane hyperplasia [28]. The difference in synovial membrane lesion may be due to prolongation of the study period (6 months). While in other species synovitis, stiffness, and marginal exostoses without damage to the articular surfaces were detected grossly or microscopically [26,27]. On the MIA group vertical fissures and erosion were observed, these in agreement with the previous studies on the horse [23,24]. There was some variability in the degree of articular cartilage changes within the same group; this may be due to imperfect correlation between body weight and carpal diameter.

Mild synovial membrane lesion in ACP group come in contrast with other studies conducted on the cartilage particle injection reported synovial fibrosis [26] with small cartilage particles engulfed inside the synovial subintimal layer [27,28]. However, other studies also reported synovial intimal hyperplasia [28]. On MIA group, presence of large cartilage particles embedded inside the subintimal layer is confirming erosion and deterioration of the cartilage surface together with leukocytic infiltration.

## 5. Recommendations

At dosage of 50 mg/joint MIA, Acute synovitis was reported at day 7 in addition to moderate degrees of articular cartilage

change at day 70. This degree of articular cartilage change generally resulted in cartilage erosion extended to deep layer and focal to diffuse gross pathologic changes in articular cartilage. On the basis of these variables, this model may be useful for evaluating anti-inflammatory drugs at the early stages (day 7) and also the disease modifying OA drugs at the late stages (day 70).

## 6. Conclusion

MIA model was successful in producing acute synovitis and joint pain in addition to degenerative joint disease. This model could be useful to those studying the pathophysiology of joint disease and may be an ideal method to test the efficacy of new drugs intended for the treatment of joint disease. ACP model need more investigation and studying of the appropriate ACP dose and the number of injections.

## 7. Authors' declaration of interests

There are no conflicts of interest.

## 8. Sources of funding

We thank Biopharm Australia for generous financial support. The work performed was not influenced at any stage by the support provided.

## References

[1] Wilsher S, Allen WR, Wood JL. Factors associated with failure of thoroughbred horses to train and race. Equine Vet J 2006;38(2):113–8.
[2] Lidner A, Dingerkus A. Incidence of training failure among thoroughbred horses at Cologne, Germany. Prev Vet Med 1993;16:85–94.
[3] McIlwraith CW. Frank Milne Lecture: From arthroscopy to gene therapy: 30 years of looking in joints. Am Assoc Equine Pract 2005;51:65–113.
[4] Pritzker K. Animal models for osteoarthritis: processes, problems and prospects. Ann Rheum Dis 1994;53:406–20.
[5] Kawcak CE, Models of Equine Joint Disease, in; Diagnosis and Management of Lameness in the Horse, Colorado, Saunders, 2011, pp. 673-677.
[6] Little CB, Zaki S. What constitutes an "animal model of osteoarthritis" e the need for consensus? Osteoarthritis Cartilage 2012;20:261–7.
[7] May SA, Animal models and other experimental system in the investigation of equine arthritis, in: Joint Diseases in the Horse, Philadelphia, Saunders, 1996, pp. 421–440.
[8] McIlwraith CW, Van Sickle DC. Experimentally induced arthritis of the equine carpus: histologic and histochemical changes in the articular cartilage. Am J Vet Res 1981;42(2):209–17.
[9] Peloso JG, Stick JA, Caron JP, Peloso PM, Soutas-Little RW. Effects of hylan on amphotericin-induced carpal lameness in equids. Am J Vet Res 1993;54(9):1527–34.
[10] Mokbel AN, El Tookhy OS, Shamaa AA, Rashed LA, Sabry D, El Sayed AM. Homing and reparative effect of intra-articular injection of autologous mesenchymal stem cells in osteoarthritic animal model. BMC Musculoskelet Disord 2011;12:259.
[11] Singh KI, Sobti VK, Roy KS. Gross an histomorphological effects of therapeutic ultrasound in experimental acute traumatic arthritis in donkeys. J Equine Vet Sci 1997;17(3):150–5.
[12] Cornelissen BP, Rijkenhuizen AB, van den Hoogen BM, Rutten VP, Barneveld A. Experimental model of synovitis/capsulitis in the equine metacarpophalangeal joint. Am J Vet Res 1998;59(8):978–85.
[13] Owens JG, Kamerling SG, Barker SA. Pharmacokinetics of ketoprofen in healthy horses and horses with acute synovitis. J Vet Pharmacol Ther 1995;18(3):187–95.
[14] Toutain PL, Cester CC. Pharmacokinetic-pharmacodynamic relationships and dose response to meloxicam in horses with induced arthritis in the right carpal joint. Am J Vet Res 2004;65(11):1533–41.
[15] de Grauw JC, van de Lest CH, Brama PA, Rambags BP, van Weeren PR. In vivo effects of meloxicam on inflammatory mediators, MMP activity and cartilage biomarkers in equine joints with acute synovitis. Equine Vet J 2009;41(7):693–9.
[16] DePuy T, Howard R, Keegan K, Wilson D, Kramer J, Cook JL, Childers MK. Effects of intra-articular botulinum toxin type A in an equine model of acute synovitis: a pilot study. Am J Phys Med Rehabil 2007;86(10):777–83.
[17] Murray RC, Janicke HC, Henson FM, Goodship A. Equine carpal articular cartilage fibronectin distribution associated with training, joint location and cartilage deterioration. Equine Vet J 2000;32(1):47–51.
[18] McIlwraith CW, Frisbie DD, Kawcak CE. Evaluation of intramuscularly administered sodium pentosan polysulfate for treatment of experimentally induced osteoarthritis in horses. AJVR 2012;73(5):628–33.
[19] Guzman RE, Evans MG, Bove S, Morenko B, Kilgore K. Mono-iodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis. Toxicol Pathol 2003;31(6):619–24.
[20] Kalbhen D. Chondroprotective and antiarthrotic properties of glycosaminoglycan polysulfate (GAGPS) in gonarthrosis in animal experiments. Z Rheumatol 1982;41:219–29.
[21] Williams J, Brandt K. Immobilization ameliorates chemically induced articular cartilage damage. Arthritis Rheum 1984;27:208–16.
[22] Williams J, Brandt K. Benoxaprofen reduces osteophyte formation and fibrillation after articular cartilage injury. J Rheumatol 1985;12:27–32.
[23] Trotter GW, Yovich JV, McIlwraith CW, Norrdin RW. Effects of intramuscular polysulfated glycosaminoglycan on chemical and physical defects in equine articular cartilage. Can J Vet Res 1989;53:224–30.
[24] Yovich JV, Trotter GW, McIlwraith CW, Norrdin RW. Effect of polysulfated glycosaminoglycan on chemical and physical defects in equine articular cartilage. Am J Vet 1987;48(9).
[25] Gustafson SB, Trotter GW, Norrdin RW, Wrigley RH, Lamar C. Evaluation of intra-articularly administered sodium monoiodoacetate-induced chemical injury to articular cartilage of horses. Am J Vet Res 1992;53(7):1193–202.
[26] Chrisman OD, Fessel JM, Stouthwick WO. Experimental production of synovitis and marginal articular exostosis in the knee joint of dogs. Yale J Biol Med 1965;37.
[27] Evans CH, Mazzocchi RA, Nelson DD, Rubash HE. Experimental arthritis induced by intraarticular injection of allogenic cartilagenous particles into rabbit knees. Arthritis Rheumatism 1984;27(2).
[28] Hurtig M. Use of autogenous cartilage particles to create a model of naturally occurring degenerative joint disease in the horse. Equine Orthop Injury Repair 1988;6:19–22.
[29] Huber MJ, Schmotzer WB, Riebold TW, Watrous BJ, Synder SP, Von Scott EA, Matthiessen PC. Fate and effect of autogenous osteochondral fragments implanted in the middle carpal joint of horses. Am J Vet Res 1992;53(9).
[30] Baxster GM, Stashak TS. Examination for lameness. In: Adams and Stashak's Lameness in Horses. Blackwell Publishing Ltd; 2011. p. 128–68.

[31] McIlwraith CW, Frisbie DD, Kawcak CE, Fuller CJ, Hurtig M, Cruz A. The OARSI histopathology initiative e recommendations for histological assessments of osteoarthritis in the horse. Osteoarthritis Cartilage 2010;18:93–105.

[32] Pritzker KPH, Gay S, Jimenez SA, Stergaard K, Pelletier JP, Revell PA, Salter D, van den Berg WB. Osteoarthritis cartilage histopathology: grading and staging. Osteoarthritis Cartilage 2006;14(1):13–29.

[33] Meachim G. Ageing and degeneration. In: AdulI Articular Cartilage. Kent: Pitman Medical; 1979. p. 215–90.

[34] Evans CH, Bowen ER, Bowen J, Tew WP, Westcott VC. Synovial fluid analysis by ferrography. J Biochem Biophys Methods 1980;2(1):11–9.

[35] Evans CH, Mears DC, Cosgrove JL. Release of neutral proteinases from mononuclear phagocytes and synovial cells in response to cartilaginous wear particles in vitro. Biochem Biophys Acta 1981;677:287–94.